Deciphering Aspartyl Peptide Sweeteners

Using the Ultimate Molecular Theory of Sweet Taste

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ABSTRACT

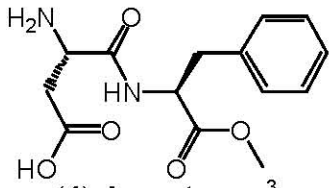
More than thirty years ago, I proposed a theory about sweet and bitter molecules' recognition by protein helical structures. Unfortunately the papers could not go to public platform until now. The sweet and bitter taste theory is updated and presented in separated papers. [1,2] Under the guidance of the sweet receptor helix recognition theory [1], aspartyl/aminomalonyl peptide sweeteners are deciphered. Here it demonstrates that, this series of sweeteners has a hydrogen-bond type hydrogen donor - hydrogen acceptor DH-B moiety and their DH-B is very special. Their B of the DH-B moiety is an oxygen of the carboxylic group, which is widely accepted one. The DH of the DH-B moiety however is the NH of the aspartyl/aminomalonyl peptide, which is a selection for the first time to the best of my knowledge. Even more unusual, their dynamic action acts through the hydrogen on α carbon of aspartyl/aminomalonyl group. The receptor main and side grooves have different space characteristics in accepting sweet molecules' groups, which is elaborated in this paper. This unprecedented elucidation well explains the aspartyl/aminomalonyl peptide sweeteners' phenomenon and, in return, strongly supports this sweet receptor helix recognition theory.

KEY WORDS: Peptide Sweetener, Hydrogen Bond/H Bridge, Intermolecular Weak Interactions, Sweet Receptor, Protein Helix, Tightening-Comeback Torsion-Spring-Like Oscillation

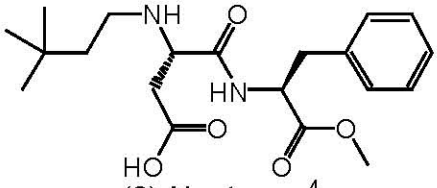
INTRODUCTION

Aspartyl peptide sweeteners were found accidentally by schlatter *et al.*[3] while working on the synthesis of the C-terminal tetrapeptide of gastrin, tryptophylmethionylaspartylphenyl-alanine amide. After the discovery of aspartyl dipeptide, thousands of derivatives were synthesized. A few of them, aspatame (**1**), neotame (**2**), advantame (**3**) and Alitame (**4**) have been developed for commercial usage.

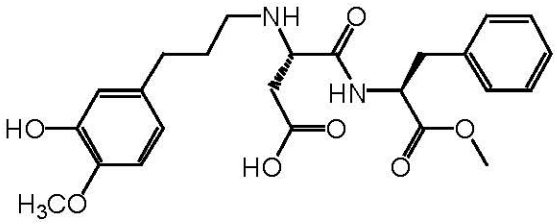
Table I Commercial Available Aspartyl Sweeteners



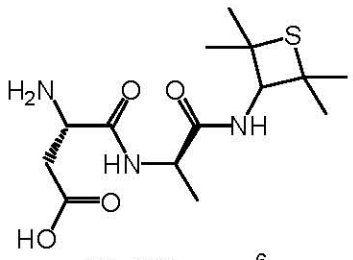
(**1**) Aspartame [3]



(**2**) Neotame [4]



(**3**) Advantame [5]



(**4**) Alitame, [6]

The rich structure-sweetness relationship information of dipeptide sweeteners greatly helped this author to form the sweet/bitter receptor protein helix recognition theory.[1,2] The sweet theory states that sweet molecules are recognized by sweet receptor protein helical structures. The recognition process is a dynamic action, in which the receptor protein helices have a torsion-spring-like oscillation movement between helical structures of 3.6 and 3 amino acids per turn.

If this theory is really successful, it should be able to decipher the structure-sweetness relationship under the guidance of this theory, and should be able to reach basically every corner of the sweet phenomenon. Also unprecedented new information may be pulled out from the analysis of the structure-sweetness relationship. In return, this should be able to support and perfect the theory.

METHODS

Where does the sweet taste theory used in this paper come from?

More than thirty years ago, upon the study of sweet/bitter-structure

relationship with different structural characteristics, the theories were formed by the combined information. Through decades' efforts, the total three papers [1,2] submitted at the same time basically described the reversal procedure for me to form the theory. As peers effectively blocked the publication for more than thirty years through peer-review processes, this allows the theories successfully evolved to current versions in an integrated form from the early version titled as Molecular Theory of Sweet and Bitter Tastes (unpublished). The contents presented here basically served as the main or initial information for the formation of these sweet and bitter theories.

Models used: The molecular models [7-9] used here are to express what the theory likes to present. In a simplified way to understand the models, you can simply treat the models as hand-drawing pictures to describe the theory. As the molecular recognition process is dynamic, the models should only be considered as a moment in the dynamic process.

RESULTS AND DISCUSSION

To accomplish the goal, three layers are arranged in the following order: First, some basic information about the characteristics of this series of compounds will be introduced. Upon this fundamental information disclosure, a binding model for this series of compounds will be established. Third, based on this binding model, further details of structure-sweetness relationships will be discussed across all of the possible corners in this series. In this way, the DH-B moiety of aspartyl peptide sweeteners, the parts acting on main grooves, side grooves, and others will be discussed in detailed manners.

1) Fundamental Characteristics Of Aspartyl/Aminomalonyl Peptide Sweeteners For Binding Model Building

The discovery of aspartame started a new era. This series of sweeteners, with clear sweet taste quality, provided not only commercial products, but also enormous insights into the secrets of the sweet taste mechanism. Aspartyl sweeteners' tremendously rich information is illustrated by their different chiral centers, conformation flexibility, groups penetrating into different receptor spaces and others. These fascinating compounds with other types of sweeteners undoubtedly prompted this author to form a theory ultimately to solve the puzzles for sweetener recognition on molecular level.

In the discussion about the bitter taste-structure relationship of denatonium compounds, [2] it was found that basically there is no critical atom or group responsible for the bitterness of its derivatives. In other words, the minimum requirement for the bitterness of denatonium derivatives is very loose. In aspartyl peptide sweeteners series, however, the situation is very different. It is therefore we start with digging out this basic information.

The initial discovery, in this series of compounds, is from aspartame. Here I would like also start from aspartame, about its replacement of both N- and C-

terminal amino acids, peptide bond manipulation, and further other groups' modification.

Manipulation of Aspartyl Group of Aspartame

More than a dozen of L-phenylalanine-containing peptide esters, in which the aspartyl group is replaced by other amino acids, including L-Glu, were synthesized and tested their taste.[3,10,11] Those dipeptides, no matter in HCl salt or free base form, are all not sweet. This series of compounds shows the importance of aspartyl group in aspartame.

Interestingly aminomalonyl group (Ama) can replace L-aspartyl and still keep the compounds sweet.[12-14] This suggested the requirements of carboxylic groups of (R)-Ama- and L-Asp- peptides for their sweet taste. Among the four diastereoisomers of Asp-Phe-OMe, only one of them (L-Asp-L-Phe-OMe) [3] shows sweet taste. Through enzymatic synthesis, Ota *et. al.* [13] showed that (R)-aminomalonyl group can provide sweet molecules, which aminomalonyl has a similar configuration as L-aspartyl group. Next let's look at the C-terminal information.

Replacement of C-Terminal Amino Acid of Aspartame

In their first paper, Mazur *et al.* [3] shows the Structure-Taste relationship of L-aspartyl dipeptide methyl esters, which L-phenylalanine in aspartame can be replaced by various amino acids.

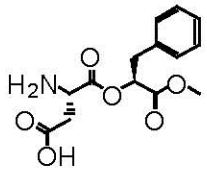
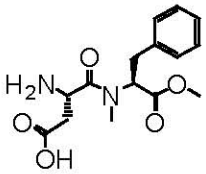
Ariyoshi[15-17] further extended the C-terminal peptide chain length. In the three series of compounds, except the pentapeptides, tri- and tetra-peptides still can provide sweet compounds. It is noteworthy that one carbon chiral change does not necessarily show sweetness loss, such as pairs of L-Asp-Gly-L-Ala-OMe & L-Asp-Gly-D-Ala-OMe, L-Asp-D-Ala-L-Ala-OMe & L-Asp-D-Ala-D-Ala-OMe, L-Asp-D-Val-L-Ala-OMe & L-Asp-D-Val-D-Ala-OMe in tripeptide series.

So far, this suggests that the C-terminal could be extended and showed more tolerance for modification. It is also see signs that one chiral center change may not necessarily have a sharp sweetness alteration. Next let's focus on aspartyl peptide bond to see its importance.

Critical Role of Peptide Bond NH

MacDonald *et al.* [18] replace aspartame's peptide NH with O (**5**) and $N(CH_3)$ (**6**). The compounds lost the sweet taste of aspartame. L-Aspartyl proline peptides do not show sweet taste (Table III), which do not behave like other similar di- or tri-peptides. The common feature for these compounds is that they lost aspartyl NH. This strongly suggested that the aspartyl peptide bond NH is very subtle and it serves as a critical role.

Table II Peptide bond-sweetness relationship of Aspartame[18]

#	Structure	Taste	#	Structure	Taste
5		NS	**6**		NS

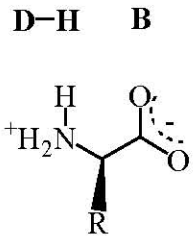
#: compound number; NS: Not Sweet

Table III Structure-taste relationship of aspartyl proline peptides[19]

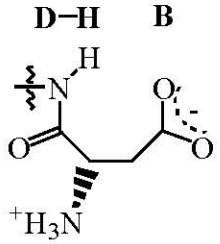
#	Compounds	Taste	#	Compounds	Taste
7	L-Asp-L-Pro-OMe	NS	**9**	D-Ala-L-Asp-L-Pro-OMe	NS
8	L-Asp-D-Pro-OMe	NS	**10**	D-Ala-L-Asp-D-Pro-OMe	NS

Determination of the DH-B and It's Type of Aspartyl Peptide Sweeteners

Having the argument above, we can settle down with one of the oxygen of the carboxylic group as B of the hydrogen donor - hydrogen acceptor DH-B moiety and the peptide bond NH as the DH. With this selection in mind, Figure I is prepared to compare their steric settlement with that of D-amino acids. The structures are drawn in a way to put the DH-B moieties on paper plane as shown in the figure. As it is generally known that D-amino acids are sweet, when comparing this two series of compounds, it is found out that the D-amino acids' R group is above paper plane and L-aspartyl peptide's amino group is below the paper plane. This means that amino acid sweeteners have non-hydrogen bond type DH-B, and L-aspartyl peptide sweeteners have hydrogen bond type DH-B moiety, according to the receptor protein helix recognition theory. To find out the supporting information for this new discovery, more information is needed for us to move forward.



D-Amino accid
Non-hydrogen bond type DH-B



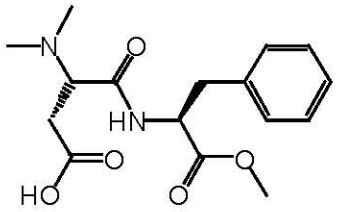
DH-B portion of L-aspartyl peptide
Hydrogen bond type DH-B required

Figure I L-Aspartyl peptide and D-Amino acid sweeteners' DH-Bs' selection and their types.

N-Substitutions on Aspartyl Amino Group.

There is a pretty peculiar compound N,N-dimethyl-L-Asp-L-Phe-OMe **(11)** (Figure II), which is not sweet.[3] This is also noticed by Nofre and Tinti[4]. The uniqueness for this compound is that there are a lot of substituted compounds still maintained their sweet taste, such as neotame (**2**), advantame (**3**) and others[19]. Also, comparing D-Ala-L-Asp-L-Phe-OMe with D-Ala-DL-Ama-L-Phe-OMe, L-Ala-L-Asp-L-Phe-OMe with L-Ala-DL-Ama-L-Phe-OMe and D-Ala-L-Asp-D-Ala-OMe with D-Ala-DL-Ama-D-Ala-OMe [19], in which aspartyl peptide shows sweet taste and aminomalonyl peptides do not have sweet taste, it can be noticed that aminomalonyl (ama) group is not simply equivalent to aspartyl, although it could replace aspartyl goup and maintain sweet taste, as shown above. Compound **(11)** provides a good insight for the understanding of aspartyl peptide sweeteners' basic characteristics, which will be further commented below.

Figure II structure and taste of N,N-dimethyl-L-Asp-L-Phe-OMe **(11)** [3]

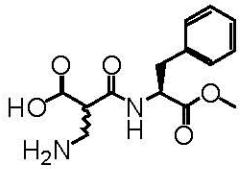
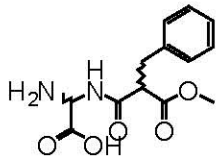
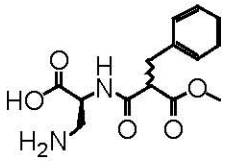


NS

Subtle Area around α-Carbon of Aspartyl/Aminomalonyl Part

There are also some other "bizarre" phenomenon that minor changes result in sweetness loss. (RS)-Ama-L-Phe-OMe peptides ((P(7.8)* 156 –P(5.9) 236X [12], 300-400X [13]), (RS)-Ama-L-β-cyclohexylalanine-OMe (300-400X[13]), (R)-Ama-(S)-Phe-OMe (800X[14]) and (R)-Ama-(S)-Phe-OEt (50X [14]) are sweet. Compound (**12**) (Table IV) however only has an amino group one carbon away from α carbon of aminomalonic acid and losses its sweet taste, although neotame (**2**) and advantame (**3**) have longer chain at the α carbon. Compound **13** and **14** have retro-inverso peptides and also no sweet taste. (*P(X) means sweetness potency comparing to X% of sucrose concentration).

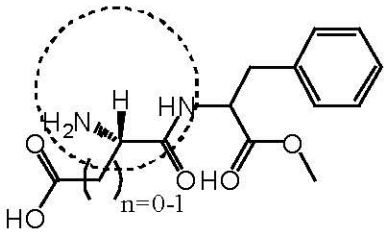
Table IV Peptide bond and Nearby Modification -Sweetness Relationship of Aspartame[18]

#	Structure	T*	#	Structure	T*	#	Structure	T*
12		NS	**13**		NS	**14**		TL

T*: Taste; TL: Tasteless; NS: Not sweet

Figure III summarizes the critical portion for aspartyl and aminomalonyl sweeteners as discussed above. Dotted circled part of aspartyl peptide is essential for the sweet taste. This part is very subtle. A little change of this portion or its surroundings will result in non-sweet molecules. Finally we can summarize the characteristics of aspartyl/aminomalonyl peptide.

Figure III Dotted circle shows the supper sensitive area of aspartyl/ aminomalonyl peptide.



Finalizing the Description about the Characteristics of Aspartyl/Aminomalonyl Peptide Sweeteners

In the path for me to search for the explanation of this H-bond type DH-B moiety thirty yeas ago, it was found that Craven and Weber [20] studied charge density in the crystal structure of γ-aminobutyric acid, a neurotransmitter, and had an important unexpected discovery. They discovered that one H atom on β carbon carries a positive charge, which forms an intramolecular bridge between negatively charged N and O atoms respectively. The magnificence of their discovery is that they provide a pinpointed detail for me to solve the essential H-bond type DH-B puzzle. The conclusion applying their unexpected discovery to our case and combining the discussions in the above-mentioned two mini-sections, is that the hydrogen on α-carbon of L-aspartic acid or aminomalonic acid peptides bridges one of the oxygen of carboxylic group and nitrogen of peptide bond (Figure IV), and makes the DH-B behave like a H-bond type.

With this information, it could be reasoned that the non-sweet compound (**11**) is because the two methyl groups on the amino nitrogen groups sterically hinder the hydrogen bridge's formation; the super sensitive area shown in Figure III is due to the electronic/steric microenvironment, distance and/or geometry requirement for this kind of H-bridge formation. X-ray structural data also demonstrated the capability for the formation of this kind of hydrogen bridge. The distances between hydrogen on α carbon and one of the carboxylic oxygen &

peptide nitrogen of aspartyl and aminomalonyl sweeteners are around 2.6 Å, such as in L-aspartyl-L-phenylalanine methyl ester hydrochloride[21] which was cited in the very first paper thirty years ago about this distance issue, alitame[22], L-aspartyl-D-alanyl-2,2,5,5-tetramethylcyclopentanyl ester[23], L-aspartyl-D-valine-(R)- α-methoxymethylbenzyl amide dihydrate[24], (R)-aminomalonyl-(S)-phenylalanine methyl ester monohydrate[25], N-3,3-dimethylbutyl-aspartyl-phenylalanine methyl ester[24], L-aspartyl-D-α-aminobutyric acid-(S)-α-ethylbenzylamide trihydrate[24] and others. We can now move on to the next step to build the binding model.

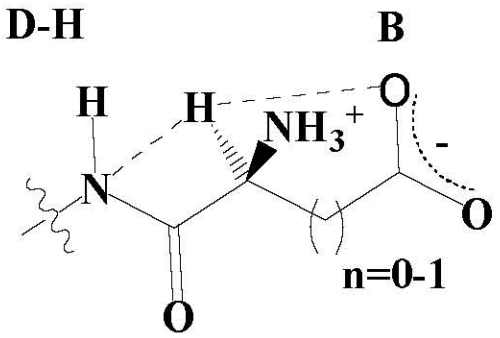


Figure IV L-Aspartyl/aminomalonyl peptide sweeteners have a special type of hydrogen-bond type DH-B. The crystal structures of L-aspartyl/ aminomalonyl sweeteners showed that distances from H on α carbon of aspartyl portion to the peptide bond nitrogen (HN···H) and to one of the carboxylic oxygen (O···H) are around 2.6 Å.[21-25]

2) Binding Model According To The Information Obtained From Above-Analyzed Results

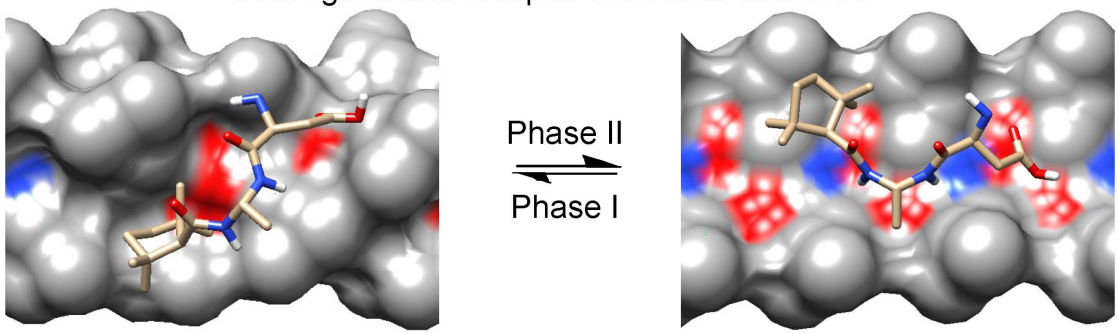
To start the building of sweeteners' binding pattern, we need to build a receptor protein helix model first. Receptor protein helix model is built up using amino acid leucine for molecular modeling purpose, which is the same as that in the first paper.[1]

Aspartyl dipeptides have a lot of rotatable σ bonds, and a lot of very complicated possible conformations. To build a reliable binding model, starting with X-ray crystal structure should be a more convincing approach. It has been shown that only L-Asp-L-Phe-OMe (aspartame) is sweet among the four possible stereoisomers. It is interesting to find out however that both of the two diastereomeric retro-inverso dipeptides N-(L-aspartyl)-N'-[(2,2,5,5-tetramethylcyclopentyl)carbonyl]-(R & S)-1,l-diaminoethane (**15**,**16**) show sweet taste.[26-28] It is pretty appealing to start from retro-inverso dipeptides due to structural requirement discrepancy for retro-inverso dipeptides comparing to "normal" aspartyl peptide sweeteners. As X-ray structure data of these retro-verso diastereomers are available, [28] the retro-inverso aspartyl R configuration

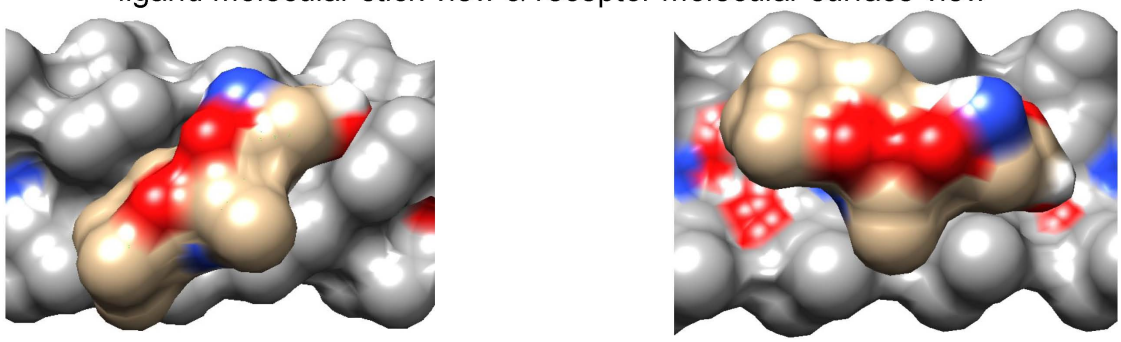
diastereomer's X-ray structure is loaded [7-9] into the receptor model and shown in Figure V.

As demonstrated in the sweet theory paper, sweeteners' recognition process is a dynamic process, in which the receptor protein helix has a torsion-spring-like oscillation between 3.6 and 3 residues of amino acids per turn. In the original status (Phase I, left column in Figure V), the helix structure is 3.6 residues of amino acids, as we know from textbook about protein α helix structure. In the final status (Phase II, right column in Figure V) of the sweet molecular recognition, every turn has three units of receptor amino acids. Figure V is a demonstration of a torsion-spring-like oscillation process during the course of sweet molecule recognition of compound (**15**). In the molecular stick views (top row), the receptor NH-O moiety in interest is highlighted by bigger diameter. The original hydrogen bonds (left column of top row) are represented using green strings. The stick view of Phase II (Final phase) of the binding model shows no hydrogen bond, which means the original hydrogen bonds are weakened or broken. In the right column, the carboxylic group and peptide NH (ligand DH-B) are almost aligned up with receptor NH-O in a complementary way. The methyl group on diamine part points at the side groove of the receptor (see receptor molecule surface views (middle and bottom of right column) of phase II). The molecule is well fitted into the main groove of the receptor. The importance of peptide DH-B moiety H-bond type is that when peptide DH-B interacts with receptor's non-H-bond type NH-O, the DH-B would extend its structure to reach receptor's NH-O; the capability for the H on α carbon of aspartyl group to bridge the carboxylic O and peptide N, which are the ligand B and D of DH-B moiety, is to bring back the extended structure and release the complementary binding of the DH-B with the receptor NH-O; in this way, the dynamic oscillation process would be kept going.

both ligand and receptor molecular stick view



ligand molecular stick view & receptor molecular surface view



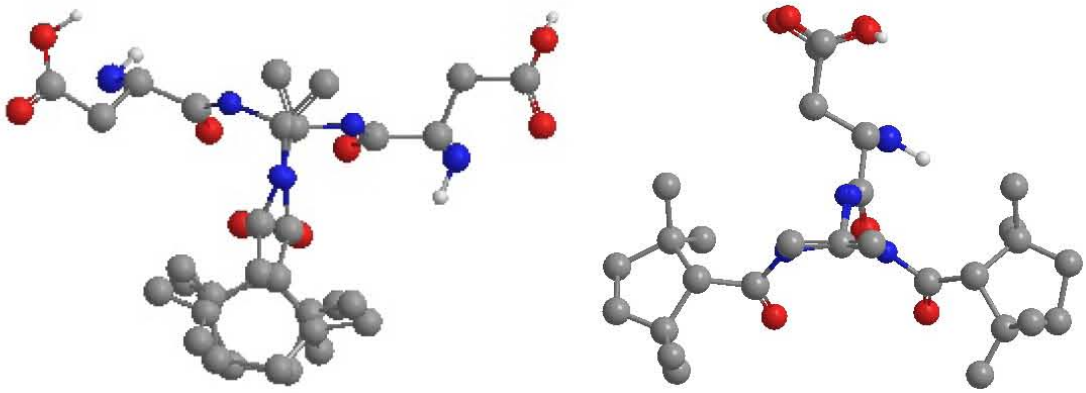
Both ligand & receptor molecular surface view

Phase I (original phase) **Phase II (Final Phase)**

Figure V X-ray structure conformation of retro-inverso dipeptide N-(L-aspartyl)-N'-[(2,2,5,5-tetramethylcyclopentyl)carbonyl]-(R)-1,l-diaminoethane (**15**) loaded on sweet receptor helix model.

Overlaps of the X-ray crystal structures[28] of the two retro-inverso diastereomers from either aspartyl or tetramethylcyclopentyl fragments are shown in Figure VI. The two conformations show pretty different orientation. The sweetness for both of the diastereomers, which phenomenon is different from aspartame series, is an unexpected finding. When this (S) configuration X-ray structure is loaded in the receptor helix, Figure VII is generated, which only show ligand stick view and receptor helix molecule surface view. This is the best available view for this conformation to be able loaded on the receptor helix. The H-bond type DH-B (peptide NH and carboxylic group respectively) of this retro-inverso S-configuration dipeptide is about perpendicular to receptor non-H bond type NH-O. This kind of binding mold may not be the real one. Then this S configuration retro-inverso dipeptide is loaded on the receptor helix again without

considering its X-ray structure, which is shown in Figure VIII. This time, this ligand DH-B aligns well with the receptor helix NH-O, and other part of the molecule fits well in the main groove of the helix. This binding conformation seems close to the NMR conformation study result. [27] This suggests that the Ariyoshi rule[29] has a conformation implication: if the molecule flexible enough and can offer a conformation which can fit to the receptor helix, the chiral center configuration of the C-end amino acid or equivalent may not be necessarily a clear-cut issue.



Overlaps of tetramethylcyclopentyl fragments

Overlaps of aspartyl fragments

Figure VI X-ray structure conformation[28] overlap images of diastereomeric retro-inverso dipeptides two diastereomers N-(L-aspartyl)-N'-[(2,2,5,5-tetramethylcyclopentyl)carbonyl]-(R & S)-1,l-diaminoethane (**15**,**16**).

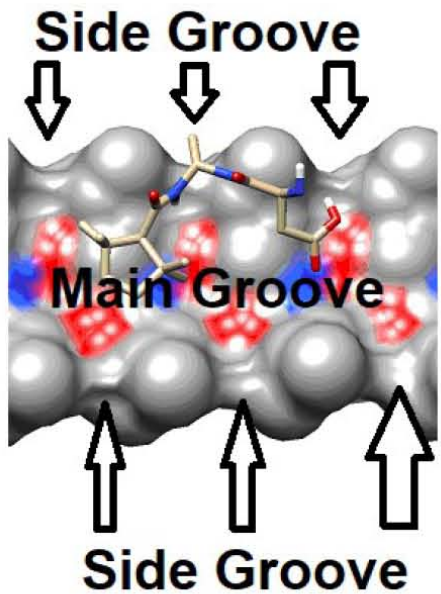


Figure VII Ligand molecular stick view and receptor helix molecular surface view of retro-inverso dipeptide N-(L-aspartyl)-N'-[(2,2,5,5-tetramethylcyclopentyl)carbonyl]-(S)-1,l-diaminoethane (**16**) X-ray crystal structure loaded on receptor helix model.

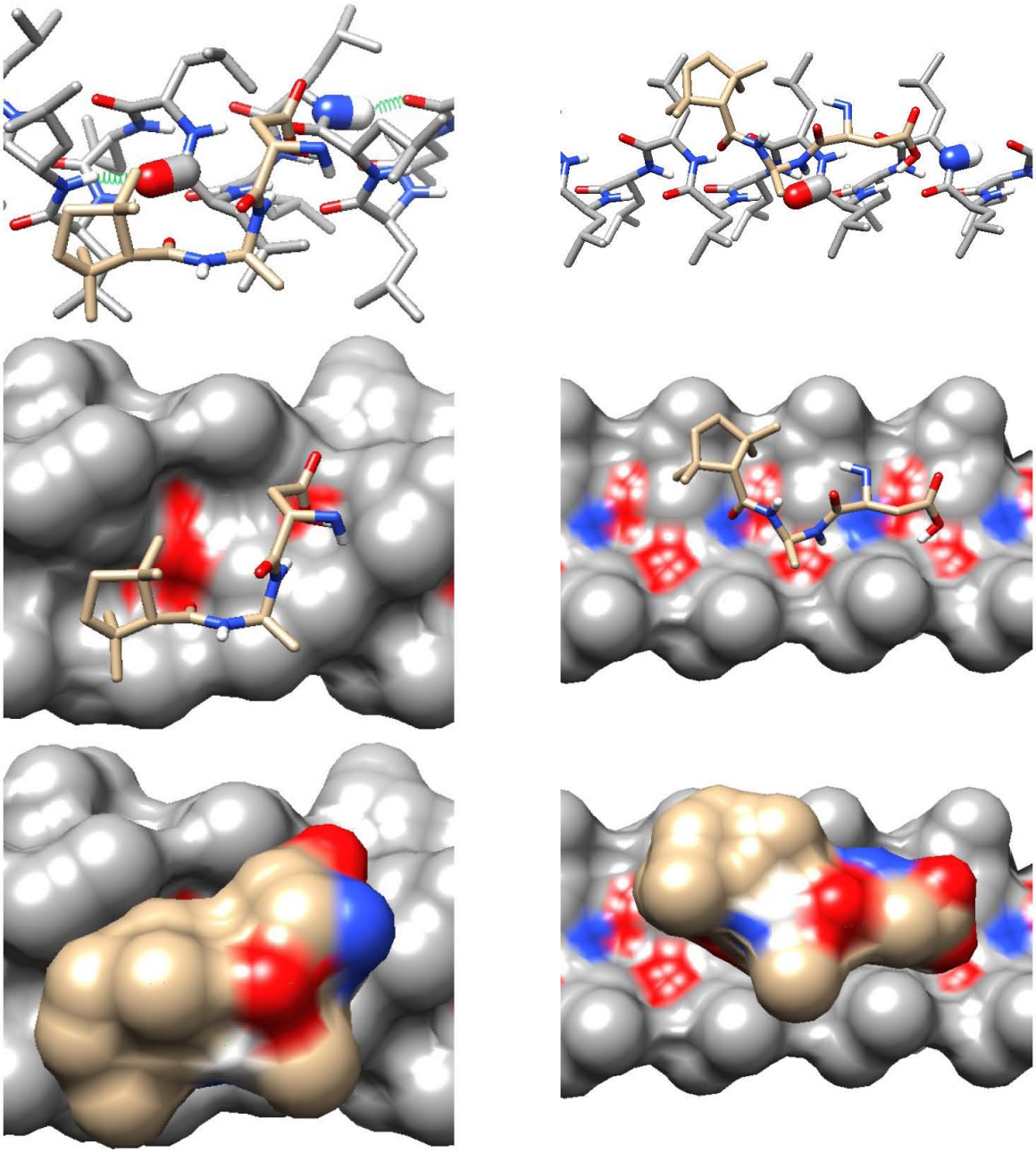


Figure VIII Retro-inverso dipeptide N-(L-aspartyl)-N'-[(2,2,5,5-tetramethylcyclopentyl)carbonyl]-(S)-1,l-diaminoethane (**16**) loaded on sweet receptor helix model without considering its X-ray crystal structure.

In summary about modeling building, the DH-B moiety, its H-bond type and the binding pattern on receptor have been established. In the next section, sweetness-structure relationship of aspartyl/Aminomalonyl sweeteners, using the information shown above, will be explained.

3) Deciphering The Structure-Sweetness Relationship Of Aspartyl Peptide Sweeteners

According to this sweet receptor protein helix recognition theory, beyond the DH-B moiety, the receptor can be roughly divided into two kinds of binding spaces: one is called main grooves, which are the ones along the helix axis direction (refer to the helix with 3 amino acids per turn and the corresponding space in the helix with 3.6 amino acids per turn); another is called side grooves, which are the ones about perpendicular to the main grooves. Following we will discuss the characteristics of aspartyl/aminomalonyl peptide sweeteners acting on both main and side grooves.

Issues about Main Grooves

Main grooves have a width which is not too much wider than one phenyl group, and the lengths are at least as long as that of tripeptide esters and very likely longer. From the above model building, it can be figured out that the aspartame analogues' C-terminal carboxylic ester group is toward the lateral side of the main groove. It is therefore when the ester's size getting bigger enough, the bigger size becomes a disadvantageous factor and the sweetness potency is getting lower (Me (**17**, 100-200X), *t*-Bu (**21**, 1X), see Table V). For groups acting on main groove chain directions, the bigger the group, the higher the hydrohobicity and the higher the sweetness potency (Me (**22**, 1X), *t*-Bu (**26**, 900X), see Table VI).

Table V Some sweetness potency of L-aspartyl L-phenyl peptide esters[3]

#	Compounds	SP	#	Compounds	SP
17	Asp-Phe-OMe	100-200			
18	Asp-Phe-OEt	10	**20**	Asp-Phe-O-*i*-Pr	1
19	Asp-Phe-OPr	1	**21**	Asp-Phe-O-*t*-Bu	1

SP: sweetness potency; Me: methyl; Et: ethyl; *i*-Pr: *iso*-propyl; *t*-Bu: *tert*-butyl

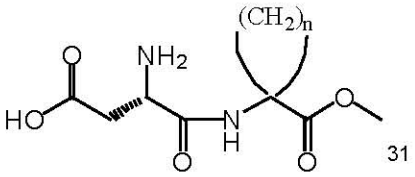
Table VI Some sweetness potency of L-aspartyl L-cysteine thioether peptide methyl esters [3,30]

#	Compounds	SP	#	Compounds	SP
22	Asp-Cys-(Me)-COOMe	1			
23	Asp-Cys-(Et)–COOMe	40	**25**	Asp-Cys-(*i*-Pr)-COOMe	170
24	Asp-Cys-(Pr)-COOMe	130	**26**	Asp-Cys-(*t*-Bu)-COOMe	900

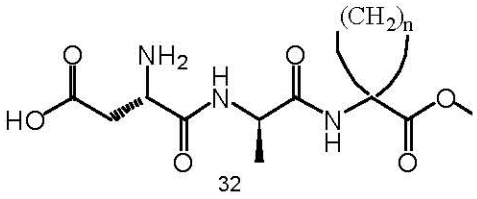
Ring size changes (Table VII) at 2nd and 3rd amino acid also probed the width of main grooves at different sites along the axis. In α-aminocycloalkanecarboxylic acid methyl esters, cyclopropane, cyclobutane, cyclopentane, cyclohexane, cycloheptane, cyclooctane ring sizes were explored. [31,32] When the ring sizes were going larger, the tastes of the compounds share very similar trend from sweet to not sweet. The only difference is that in L-aspartyl-α-aminocycloalkanecarboxylic acid methyl esters series, the taste changes from six-membered ring (**30**) and in L-Asp-D-Ala-AA-OCH_3 series, the

taste changes from seven-membered ring (**37**). The trend is due to the width of the main groove. When the acting portion pushed to the limits at the width of main groove, the sweetness potency will loss and eventually disappear.

Table VII Ring size-sweetness relationship of L-aspartyl peptides



31



32

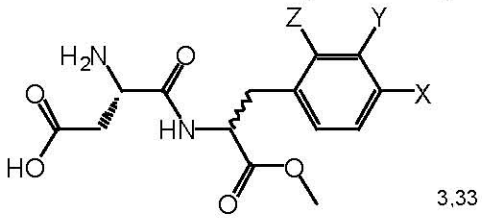
Ring size	#	Taste	#	Taste
cyclopropane	**27**	S	**33**	S
cyclobutane	**28**	S	**34**	S
cyclopentane	**29**	S	**35**	S
cyclohexane	**30**	bitter	**36**	S
cycloheptane	**31**	Very bitter	**37**	bitter
cyclooctane	**32**	TL	**38**	bitter

S: Sweet; NS: Not Sweet; TL: Tasteless.

Substituent's Effect on Aromatic Phenyl Ring When Acing on Main Groove

Table VIII shows Structure-Sweetness relationship of L-Asp-substituted Phe-OMe. Among the hydroxyl and/or methoxy mono- and/or di-substituted compounds, only para-methoxy substituted derivative (**54**) sustained the aspartame's sweetness potency. This is an important piece of information which states that this is different from the similar groups acting on receptor helix side grooves, which will be discussed later. Substituents on groups acting on side groove could increase sweetness potency tremendously.

Table VIII Structure-Sweetness relationship of L-Asp-substituted Phe-OMe



3,33

#	Phe Config.	X	Y	Z	Taste
39	L	H	H	H	80-200
40	D	H	H	H	NS
41	DL	H	H	H	80-200
42	L	OH	H	H	10-40
43	D	OH	H	H	TL
44	DL	OH	H	H	10-40
45	L	OMe	H	H	1-5
46	L	H	OH	H	10-40
47	D	H	OH	H	TL
48	DL	H	OH	H	10-40
49	L	H	OMe	H	10-40
50	DL	H	OMe	H	10-40
51	L	H	H	OH	10-40
52	D	H	H	OH	TL
53	DL	H	H	OH	10-40
54	DL	H	H	OMe	80-200
55	L	OH	OH	H	1-5
56	L	OMe	OH	H	TL
57	L	OH	OMe	H	1-5
58	L	OMe	OMe	H	TL

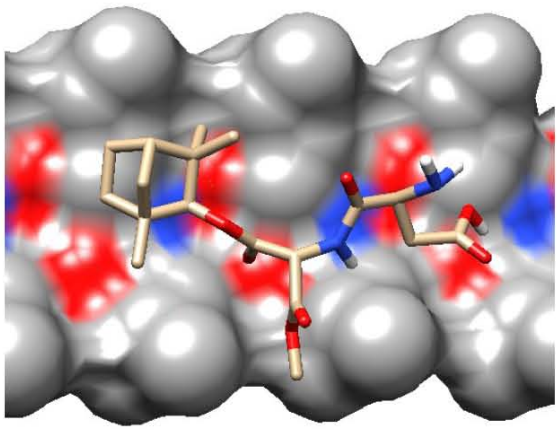
Rigidity is definitely helping the group perturbing sweet receptor helix to perform its torsion-spring-like oscillation process. Among the huge number of aspartyl peptide sweeteners' C-terminal substituents, the best example probably is the fenchyl group. Table IX lists the four fenchyl isomers' sweet potency data. The capability to increase sweetness potency decreases in the order of (+)-β-Fn, (-)-α-Fn, (-)-β-Fn, (+)-α-Fn. Their possible binding patterns are shown in Figure IX to attempt to explain the sweetness sequence. As the binding is on a shallow groove, hydrophobicity plays a critical role here. The $-CH_2CH_2-$ part of the best two fenchyl groups (+)-β-Fn, (-)-α-Fn is sitting on bottom (Figure IX). Correspondingly, for (-)-β-Fn, (+)-α-Fn dipeptide sweeteners, there is only $-CH_2-$ part sitting on the bottom. The binding patterns of three methyl groups in (-)-β-Fn, (+)-α-Fn resemble to those in (+)-β-Fn, (-)-α-Fn respectively. This probably is the reason for this series of isomers divided into two groups for the sweetness and having the sweetness sequence showing in Table IX. So far, basically the description about peptide sweetener groups acting on main grooves is

completed. We can move to peptide sweetener groups acting on side grooves now.

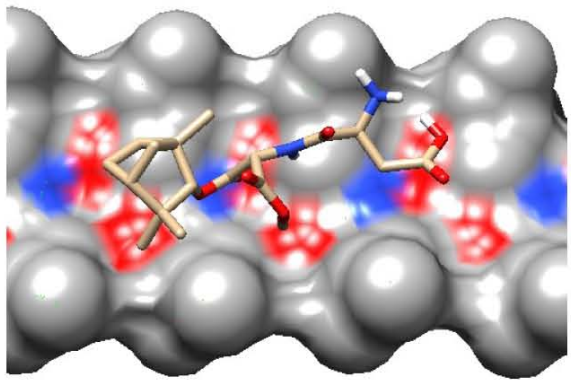
Table IX. Sweetness potency of L-AspNH-CH(R)COOFn

	#	R H[34]	#	R D-Me[34]	#	R DL-CO_2Me[34]	#	R D-Phenyl[35]
O-(+)-α-Fn	**59**	0	**63**	0	**67**	1,000	**71**	220 ± 40
O-(-)-β-Fn	**60**	0	**64**	180	**68**	5,000	**72**	480 ± 160
O-(-)-α-Fn	**61**	60	**65**	600	**69**	30,000	**73**	1,200 ± 300
O-(+)-β-Fn	**62**	600	**66**	2,600	**70**	50,000	**74**	3,700 ± 1,100

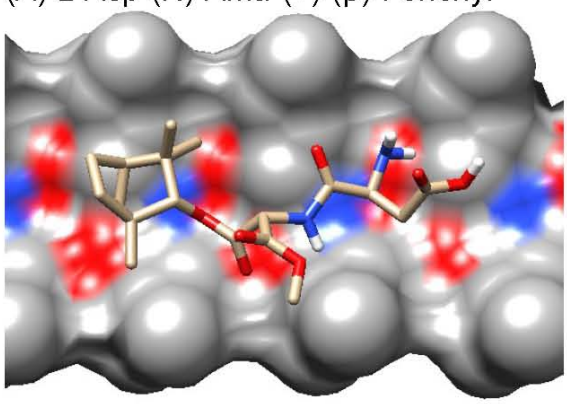
Fn: Fenchyl



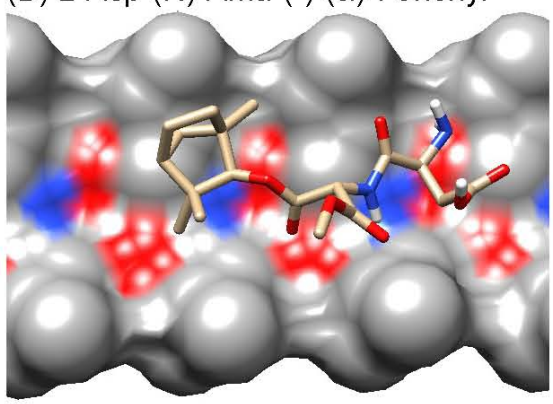
(A) L-Asp-(R)-Ama-(+)-(β)-Fenchyl



(B) L-Asp-(R)-Ama-(-)-(α)-Fenchyl



(C) L-Asp-(R)-Ama-(-)-(β)-Fenchyl



(D) L-Asp-(R)-Ama-(+)-(α)-Fenchyl

Figure IX. L-Aapartyl-(R)-Aminomalonyl Fenchyl Dipeptide Sweeteners docked into receptor protein helix with 3 amino acids per turn (Phase II of the torsion-spring-like oscillation) for possible binding models to explain the fenchyl structure-sweetness relationships.

Issues about Side Groove

Due to the side grooves having a narrower space than the main grooves, which can be seen from molecular surface view of phase II of above ligand-receptor models, ligand groups need to be very narrow. It is also due to the movement of the side chains on both sides of the grooves, which make the side chains have a movement like "sliding" on the ligand group's surface. It is therefore the groups need to be flat planes, in which aromatic rings undoubtedly are perfect candidates. As to which kind of substituents will enhance the sweet potency, there is a need to introduce the probably not many people noticed research area about intermolecular weak interactions in a brief manner. Knowing the results in that research area will definitely help our discussion in the structure-sweetness relationship.

Intermolecular Weak Interactions

Having been discussed above, the ligand groups acting on the space of side grooves of receptor need to be flat moieties. Indeed what happened is aromatic rings played a great roles in enhancing sweetness potency. When flat aromatic groups appear in sweeteners, they could be called serving as π-stacking/pi-interaction [36-39] to explain these groups' attribution for the sweetness. The π-stacking/pi-interactions have been known for a long time and there is huge number of publications dealing with these issues. [40-45] There are a couple of results worthy to give a short summary.

Hunter and Sanders[40] noticed that electron donor-acceptor concept can be misleading about so-called pi interaction and it is the properties of the atoms at the points of intermolecular contact rather than the overall aromatic ring property. Substituents on aromatic ring no matter electron-donating or accepting groups provide stronger σ-π or π- π interaction. [42-44,46] Martinez and Iverson[41] suggested that pi-stacking/pi interactions may imply face-centered stacking arrangements, which may not be true in most of situations, it is therefore the term should only be used in a restricted manner. [45]

Translating the information into our topic is that sweet receptor side chains do not necessarily have to have any aromatic group at binding sites; secondly, both electron-donating or accepting groups could enhance binding, which means increasing sweetness potency for our concern here. The latter piece of information is critical for our following structure-sweetness relationship discussion.

Issues about Substituent Effects Acting on Side Groove and/or Nearby

Figure X shows N-[3-(3-hydroxy-4-methoxyphenyl)propyl]-α-L-aspartyl-(1R,2S,4S)-1-methyl-2-hydroxy-4-phenylhexylamide (**75**, 15,000 times more potent than sucrose)[47] loaded onto receptor model. The DH-B, its moving pattern, its hydrogen bonds behavior, the torsion-spring-like oscillation process are the same as discussed above. One methyl group is pointing at the lower side

groove and one ethyl group is pointing at the upper side groove. The 3-(3-hydroxy-4-methoxyphenyl)propyl group is our focus in this section. In this molecule, the 3-hydroxy-4-methoxyphenyl group is acting on the space of side grooves and three carbon chain is the linker. The two side chains on both sides of the side groove have a zigzag movement during the recognition process. The side chains have a relative movement like sliding on the plane of 3-hydroxy-4-methoxyphenyl group.

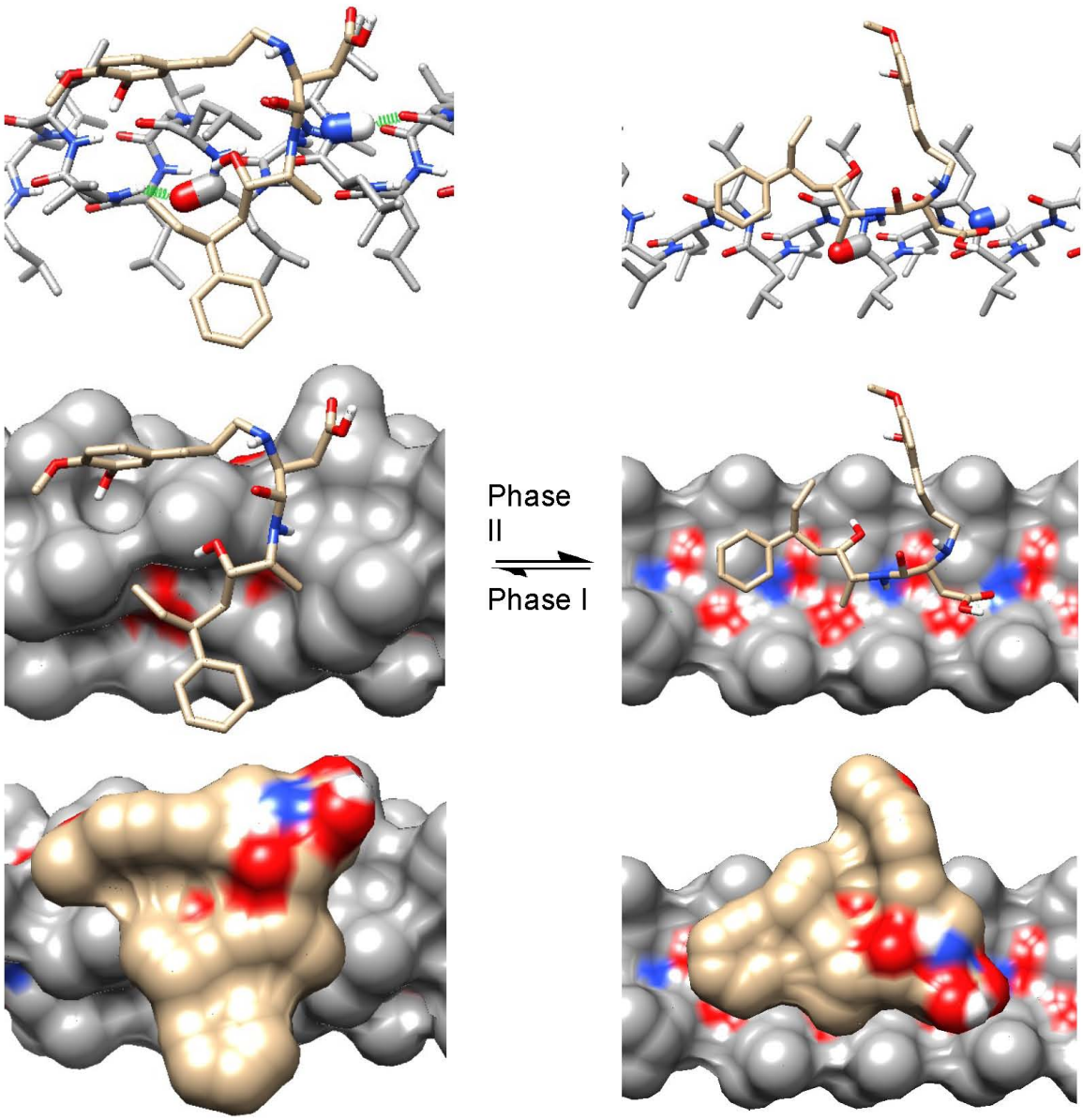


Figure X Compound (**75)** docking diagram on receptor model.

It is interesting to notice that the major peptide chain and the N-[3-(3-hydroxy-4-methoxyphenyl)propyl] parts of the conformation in phase II (Figure X)

share a similar conformation with its X-ray structure[47]. The difference between the two conformations is that the phenyl group is pointing at different direction (Figure XI), which is very similar to the above discussed retro-inverso analogs.

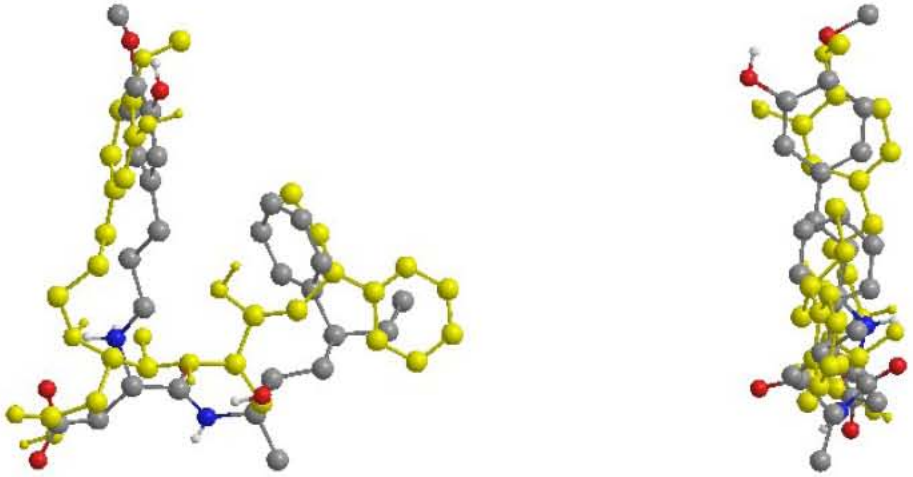
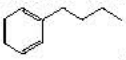
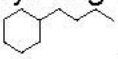


Figure XI Overlap of docked conformation in phase II of Figure X with X-ray crystal structure[47] of N-[3-(3-hydroxy-4-methoxyphenyl)propyl]-α-L-aspartyl-(1R,2S,4S)-1-methyl-2-hydroxy-4-phenylhexylamide (**75**). The yellow molecule is the conformation docked to the receptor helix model.

There are a lot of compounds synthesized, which probed side groove. [4,48-55] A few selected compounds for this section's purpose will be discussed.

For compounds (**76**) ($CH_3CH_2CH_2CH_2$ 400X), (**77**) ($(CH_3)_2CHCH_2CH_2$, 1,300X) and (**2**) ($(CH_3)_3CHCH_2CH_2$, Neotame, 10,000X, neotame), every CH_2 increment (increase of hydrophobicity) increases sweetness potency. When the chain goes longer with a phenyl ring, it still shows sweet taste (**78**,  , 1,500[4], 1000X[5]). When the phenyl ring is reduced to a cyclohexyl ring, the sweetness taste disappears (**79**  , tasteless). It is because the phenyl group has reached the side groove territory and this narrow side groove only allows a flat planar group.

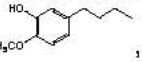
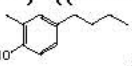
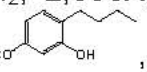
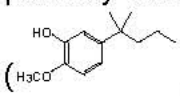
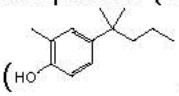
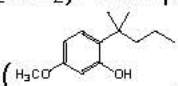
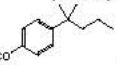
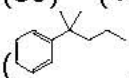
With the substituents on the aromatic ring, the sweetness potency increases as discussed in intermolecular weak interaction section. Compounds (**80**) ($(3\text{-}OCH_3,4\text{-}OH)C_6H_5CH_2CH_2CH_2$, 2,500X), (**3**) ( , 20,000X), (**81**) ( , 35,000X), and (**82**) ( ,15,000) have higher sweetness potency than unsubstituted compound (**78**) ($C_6H_5CH_2CH_2CH_2$). Compounds (**83**) ( , 50,000X), (**84**) ( , 50,000X), (**85**) ( , 20,000X), (**86**) ( ,20,000X) have higher sweetness potency than (**87**) ( ,4,000X). These substituents are CH_3, OH CH_3O, which are electron-donating groups and they provided sweetness enhancement effect.

Table X N-substituted aspartame

4,5

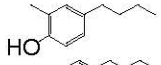
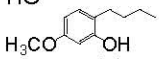
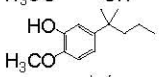
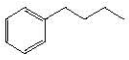
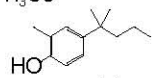
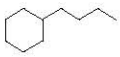
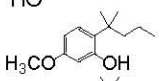
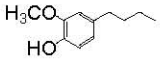
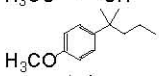
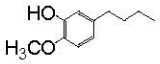
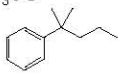
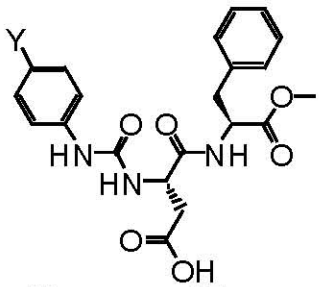
#	R	SP	#	R	SP
76	$CH_3CH_2CH_2CH_2$	400	**81**		35,000
77	$(CH_3)_2CHCH_2CH_2$	1,300	**82**		15,000
2	$(CH_3)_3CCH_2CH_2$	10,000	**83**		50,000
78		1,000	**84**		50,000
79		0	**85**		20,000
80		2,500	**86**		20,000
3		20,000	**87**		4,000

Table XI[51] shows a series of N-substituted aspartame urea compounds. There are a series of electron-withdrawing group on the urea aromatic ring. Compounds with substituents of O_2N (**88**), NC (**89**), C_2H_5OCO (**90**), CH_3CO (**91**), CH_3SO_2 (**92**), CH_3OCO (**93**), Cl (**94**) have sweetness potency 14,000X, 10,000X, 2,400X, 2,200X, 2,100X, 2,000X, 200X respectively, which are more potent than the corresponding unsubstituted compound (**95**, Y=H, 130X). The compounds in Tables X and XI have a very similar structure. The difference between them is the linker $-CH_2CH_2CH_2-$ and –NHCONH-. The sweetness enhancing effect of electron-donating groups in Table X and electron-withdrawing groups in Table XI is well explained with the intermolecular weak interaction information, which has been discussed above.

Table XI Aspartame urea compounds[51]



#	Y	SP	#	Y	SP
88	O_2N	14,000	**92**	CH_3SO_2	2,100
89	NC	10,000	**93**	CH_3OCO	2,000
90	C_2H_5OCO	2,400	**94**	Cl	200
91	CH_3CO	2,200	**95**	H	130

CONCLUSION

More than thirty years ago, I proposed a theory about sweet and bitter molecules' recognition by protein helical structures. Unfortunately the papers could not go to public platform until now. Both of the theories are updated.[1,2] The two theories are mainly initiated from the contents presented in this paper, although there are a lot of information presented in other papers or even not presented in these papers. The processes for the theories' formation are basically the reversal procedures for the information presented in these papers. The total three papers are independent and as a whole make the foundation for the theories very strong.

Under the guidance of the sweet receptor protein helical structure recognition theory, the deciphering of aspartyl/aminomalonyl peptide sweeteners is started with identifying the characteristics of this series of sweeteners. Upon the sorting out of the information from the available structure-sweetness relationship, the hydrogen donor - hydrogen acceptor DH-B is identified as the free carboxylic group of aspartyl/aminomalonyl group as B and the peptide bond NH as DH. Surprisingly this series of sweeteners has a H-bond type DH-B. Even more astonishingly, this H-bond type of DH-B behaves through the hydrogen on α carbon of aspartyl/aminomalonyl part, which bridges the D and B of the DH-B. No matter the selection of the DH-B, or the identification of H-bond type DH-B, or the revealing of its action through the hydrogen on α carbon of aspartyl/aminomalonyl part, all of the information is disclosed unprecedentedly. These discoveries are only possible with the guidance of this sweet receptor protein helix recognition theory.

Upon the identification of the characteristics of this series of sweeteners, binding models of a few representative sweet molecules are established, in which the sweet molecules are well fitted in the receptor model. The two kinds of binding spaces (main grooves and side grooves) are discussed in detailed manner. As the grooves are very shallow, hydrophobicity is the basic property for enhancing the sweetness potency. The main grooves' width and depth in axis direction are discussed with examples.

Overwhelmingly important information is also extracted from details related to side grooves with the help of this theory. As the gap of the side groove is very narrow in the helix with 3 amino acids per turn and side chains on both sides of side groove has a zigzag movement in the recognition process according to this theory, the groups acting on the side groove need to be flat plane, which usually are aromatic rings. This prompts author to search information about so-called pi-interaction. From there, we reached to the research information about so-called intermolecular weak interactions. Combining information about intermolecular weak interactions, sweeteners' groups acting on side grooves and this sweet receptor helix recognition theory, we reached very important conclusion: receptor binding site does not necessarily have aromatic ring; both electron-donating and -withdrawing groups acting on side grooves could enhance sweetness potency.

Through the process of deciphering aspartyl/aminomalonyl peptide sweeteners, sweet receptor protein helix recognition process for sweet molecules, which involves a torsion-spring-like oscillation movement between helical structures of 3.6 and 3 amino acids per turn (tightening-comeback torsion-spring-like oscillation), is well elucidated. This deciphering process not only deciphers the structure-sweetness relationship of aspartyl/aminomalonyl peptides in an unprecedented manner, but also further perfects this sweet taste theory elucidation.

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CONFLICT OF INTEREST

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